LC-MS quantitation of retatrutide: triple receptor agonist for obesity and diabetes treatment

Sujata Rajan[1], Sashank Pillai[1], Eshani Galermo[2] and Rahul Baghla[2]
[1]SCIEX, India and [2]SCIEX, USA

This technical note demonstrates a sensitive method for the quantitation of retatrutide in human plasma using Zeno MRM^{HR} on the ZenoTOF 7600 system. A lower limit of quantitation (LLOQ) of 2.5 ng/mL was achieved with simple and fast sample preparation (Figure 1).

The increasing prevalence of obesity and type 2 diabetes mellitus (T2DM) presents a major global health challenge, as many existing therapies lack adequate efficacy and safety.[1] As a triple-action agonist, retatrutide enhances satiety, suppresses hunger and boosts energy expenditure, potentially providing superior benefits for blood sugar control and weight loss compared to other incretin-based therapies.[2] Based on preclinical data, retatrutide has shown a promising approach to addressing these unmet clinical needs. Therefore, when assessing levels of retatrutide in biological matrices for toxicokinetic and pharmacokinetic profiles, it is crucial to employ sensitive assays to help effectively evaluate the efficacy and the safety of the drug.

Key benefits for analysis of retatrutide using the ZenoTOF 7600 system

- **Sensitive quantitation of retatrutide:** Achieve 2.5 ng/mL LLOQ for the quantitation of retatrutide in human plasma
- **Simple and fast sample preparation:** Utilize a simple protein precipitation and achieve an average 89.6% recovery for the quantitation of retatrutide in human plasma
- **Robust analytical performance:** Achieve highly reproducible quantitative performance with %CV <10 at all concentration levels across a linear dynamic range (LDR) of 3.6 orders of magnitude
- **Streamlined data management:** SCIEX OS software, a 21 CFR Part 11-compliant platform, simplifies data acquisition and processing

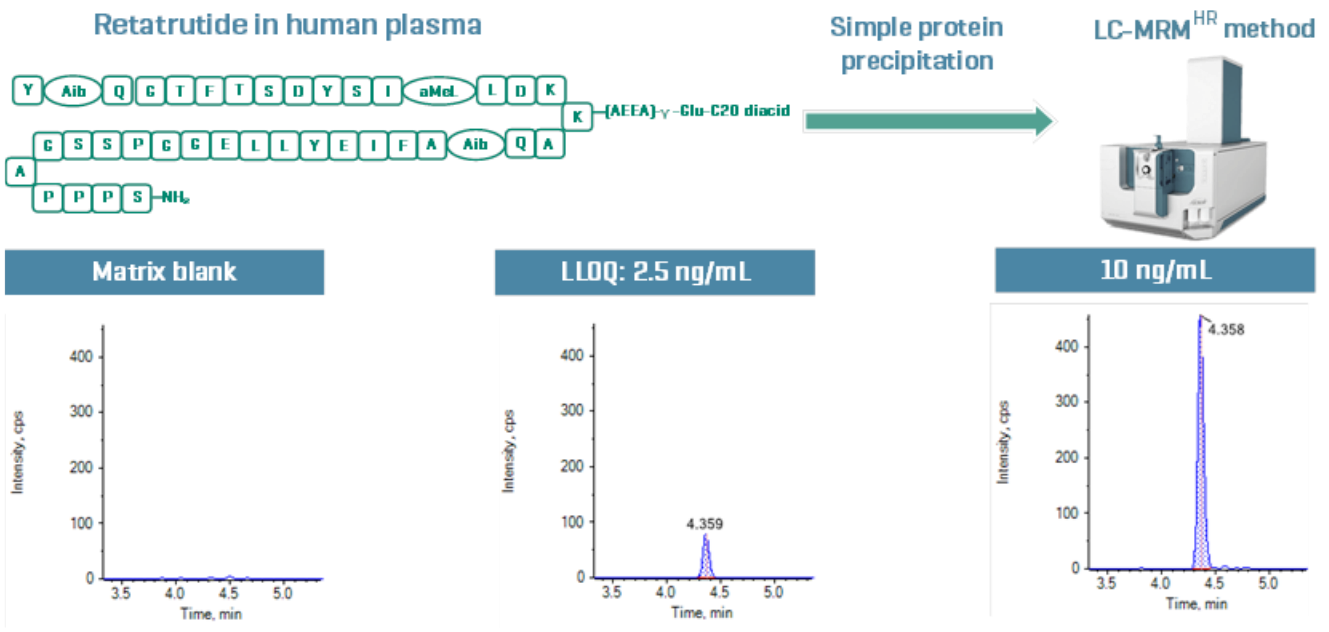

Figure 1. Representative extracted ion chromatograms (XICs) for retatrutide in extracted human plasma at matrix blank, 2.5 ng/mL (LLOQ) and 10 ng/mL. No interferences were observed in the matrix blank.

Introduction

Retatrutide (LY3437943 or LY) is a 39-amino acid peptide engineered from a GIP backbone to act as a triple agonist at the glucagon (GCGR), GIP (GIPR), and GLP-1 (GLP-1R) receptors.[3] The synergistic receptor activation enhances insulin secretion, improves glucose regulation and suppresses appetite, leading to better glycemic control and substantial weight loss. Clinical trials have shown significant efficacy, with marked reductions in body weight and improved glycemic outcomes, positioning retatrutide as a promising treatment for obesity and type 2 diabetes. Additionally, retatrutide has shown potential in lowering cardiovascular risk and treating non-alcoholic fatty liver disease, highlighting the broader impact on metabolic health.[4]

Due to its high efficacy in treating T2DM and obesity along with improvement of cardiovascular health, highly sensitive assays are necessary to ensure precise and accurate detection and quantitation when assessing the pharmacokinetic and pharmacodynamic effects.

Methods

Standard preparation: 1 mg of retatrutide standard was weighed and dissolved in 75:25 (v/v) methanol:water, with 0.1% formic acid as diluent.
Calibration curves (2.5–10,000 ng/mL) were prepared in human plasma by serial dilution and analyzed in triplicate. Pre- and post-spiked plasma samples (in 200 µL of human plasma) were prepared at 2.5, 7, 4000 and 8000 ng/mL, with each concentration analyzed in triplicate.
Sample preparation: Retatrutide was spiked into 200 µL of human plasma (2.5–10,000 ng/mL). Each sample was mixed with 600 µL of ice-cold methanol containing 0.1% formic acid, vortexed for 3 min at 2500 rpm, and centrifuged at 15,000 rpm for 5 min. A 100 µL aliquot of the supernatant was transferred to low-binding vials for analysis.
Chromatography: Analytical separation was conducted on an ExionLC 40 system with a Phenomenex Kinetex C8 column (2.1 × 100 mm, 2.6 µm) at 0.3 mL/min. Mobile phase A and B were 0.1% formic acid in water, and 0.1% formic acid in acetonitrile, respectively. The column was kept at 40 °C. Gradient conditions are summarized in Table 1. A 5 µL sample was injected for LC-MS analysis. Flow was diverted to waste from 0–3.5 min and 5–9 min to prevent contamination of the mass spectrometer.
Mass spectrometry: The optimized source and gas parameters are listed in Table 2 and the Zeno MRM^{HR} parameters are included in Table 3.
Data processing: Analysis was performed using SCIEX OS software, version 3.4.5. Peaks were integrated using the MQ4 algorithm, and a weighting of $1/x^2$ was used for retatrutide quantitation. An XIC peak width of 0.02 Da was applied.

Table 1. LC gradient conditions for retatrutide.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	95	5
6.0	20	80
7.0	20	80
7.2	95	5
9	95	5

Table 2. Source, gas and ZenoTOF 7600 system conditions.

Parameter	MS	MS/MS
Scan mode	TOF MS	MRM^{HR}
Polarity	Positive	
Gas 1	80 psi	
Gas 2	70 psi	
Curtain gas	35 psi	
Source temperature	650°C	
Ion spray voltage	5500 V	
CAD gas	12	
CE	10	*See table 3*
Start mass	m/z 500	m/z 250
Stop mass	m/z 1250	m/z 1250
Q1 resolution	NA	Unit
Accumulation time	0.2 s	0.2 s
Zeno trap	NA	ON
ZOD threshold (CID)	NA	20,000 cps
Time bins to sum	8	8

Table 3. Zeno MRM^{HR} parameters used for quantitation.

ID	Precursor ion (m/z)	Fragment ion (m/z)	CE (V)	DP (V)
Retatrutide_01	1183.88	396.2189	50	25
Retatrutide_02	1183.88	795.4069	50	25

Quantitative performance on ZenoTOF 7600 system

A triplicate injection was performed across concentrations ranging from 2.5 ng/mL to 10,000 ng/mL (Figure 2). An LDR of 3.6 orders of magnitude was achieved. A weighing factor of $1/x^2$ was used and a coefficient of determination (r^2) of >0.996 was reached, showing excellent linearity across a wide calibration range.

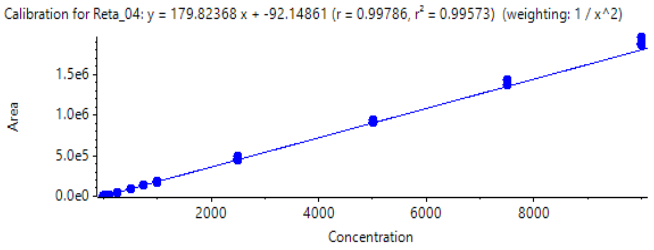

Figure 2. Calibration curve for the quantitation of retatrutide in human plasma with a weighting factor of $1/x^2$. An LDR of 3.6 orders of magnitude was achieved with a r^2 >0.996.

Analytical performance was evaluated based on the requirement that the accuracy of the calculated mean should be between 80% and 120% at the LLOQ and between 85% and 115% at higher concentrations. The %CV of the calculated mean of the concentration should be below 20% at the LLOQ and below 15% at all higher concentrations.[4]

The assay accuracy was within ±13 % of the nominal concentration and the %CV was <10 for the quantitation of retatrutide in human plasma (Figure 3). Calculated %accuracy and %CV values were within the acceptance criteria at each concentration level.

Recovery was evaluated for retatrutide at 4 different concentrations (2.5, 7, 4000 and 8000 ng/mL). A triplicate injection of the pre-spiked samples was compared to the post-spiked samples. The average recovery was 89.6% with a %CV of 4.5, achieved using a simple protein precipitation approach (Table 4).

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates
4	Reta_04	2.500	3 of 3	2.544	0.245	9.62	102.
5	Reta_04	5.000	3 of 3	5.018	0.102	2.04	100.
6	Reta_04	10.000	3 of 3	9.818	0.606	6.17	98.2
7	Reta_04	25.000	3 of 3	21.836	0.979	4.48	87.3
8	Reta_04	50.000	3 of 3	47.590	1.099	2.31	95.2
9	Reta_04	100.000	3 of 3	100.193	5.755	5.74	100.
10	Reta_04	250.000	3 of 3	254.480	5.615	2.21	102.
11	Reta_04	500.000	3 of 3	490.448	16.940	3.45	98.1
12	Reta_04	750.000	3 of 3	776.062	25.364	3.27	103.
13	Reta_04	1000.000	3 of 3	997.733	38.813	3.89	99.8
14	Reta_04	2500.000	3 of 3	2590.047	116.626	4.50	104.
15	Reta_04	5000.000	3 of 3	5106.087	81.777	1.60	102.
16	Reta_04	7500.000	3 of 3	7707.983	200.772	2.60	103.
17	Reta_04	10000.000	3 of 3	10536.596	288.033	2.73	105.

Figure 3. Quantitative performance for retatrutide (m/z 1183.8→ m/z 396.2) analysis. Reproducibility and accuracy results were determined from the calibration curve standards across 3 replicates at each concentration. Statistical results were summarized using the Analytics module in SCIEX OS software.

Table 4. Recovery was evaluated using 2.5 ng/mL, 7 ng/mL, 4000 ng/mL and 8000 ng/mL of retatrutide. Each concentration was evaluated in triplicate injections.

Recovery			
Concentration (ng/mL)	Area response in pre-spiked samples (Mean area ± SD)	Area response in post-spiked samples (Mean area ± SD)	%Recovery
2.5	332±17.3	394±35.6	84.4
7	1262±20.3	1415±101.5	89.2
4000	675954±30754	759888±23484.5	89.0
8000	1241777±12614.5	1296914±24666.8	95.7
		Average recovery (%)	89.6
		Standard deviation	4.04
		%CV	4.5

Compliance-ready SCIEX OS software

Equivalent SCIEX OS software capabilities for regulated bioanalysis can be executed on the ZenoTOF 7600 system, ensuring high fidelity when performing method transfers while retaining critical compliance features. SCIEX OS software is a closed system and requires records and signatures to be stored electronically, meeting the regulations outlined by 21 CFR Part 11. SCIEX OS software can open raw data files from any visible storage location within a closed network by using designated processing workstations.

Figure 4 illustrates the features of SCIEX OS software used to monitor the audit trail, acquire and process data, and configure user access. The audit trail feature enables users to audit critical user actions and locks in data integrity. The Central Administrator Console (CAC) feature allows users to centralize acquisition and processing using a single platform to maximize efficiency for multi-instrument laboratories, independent of compliance standards.

The configuration module allows users to assign roles and access as the administrator, method developer, analyst and reviewer.

Figure 4. Features of SCIEX OS software for monitoring user access and evaluating the audit trail. The audit trail view allows users to filter for high-risk events easily and enables data integrity features to meet compliance requirements. The software features a Central Administrator Console (CAC) to manage users and groups, role definitions, workstations and projects across all systems. The CAC feature supports both regulated and non-regulated compliance standards. The configuration module enables users to quickly set up roles and levels of access for the administrator, method developer, analyst and reviewer levels.

Conclusions

- An LOQ of 2.5 ng/mL was achieved for the quantitation of retatrutide in human plasma
- Linearity was achieved at concentrations ranging from 2.5 ng/mL to 10,000 ng/mL with an r^2 >0.996 covering an LDR of 3.6 orders of magnitude
- Good quantitative performance was demonstrated with accurate and highly reproducible (%CV <10) results on the ZenoTOF 7600 system
- An average recovery of 89.6% was achieved with %CV <4.5 using a simple sample preparation
- Retain data management and compliance-readiness (21 CFR Part 11) features using SCIEX OS software to support quantitative analysis on the ZenoTOF 7600 system

References

1. Glucagon-like peptide 1-based therapies for the treatment of type 2 diabetes mellitus, Kathleen Dungan, David M Nathan, Katya Rubinow, Feb 2025.
2. Peptide Sciences, Retatrutide, Dr. Hanna
3. LY3437943, a novel triple glucagon, GIP, and GLP-1 receptor agonist for glycemic control and weight loss: From discovery to clinical proof of concept, Tamer Coskun *et. al.* August 2022
4. The power of three: Retatrutide's role in modern obesity and diabetes therapy. Volume 985, 15 December 2024, 177095 Toufik Abdul-Rahman *et. al.*
5. Bioanalytical Method Validation, May 2018
6. Triple–Hormone-Receptor Agonist Retatrutide for Obesity — A Phase 2 Trial, Ania M. Jastreboff *et. al.* Vol 389 no 6, June 26 2023

Headquarters
250 Forest Street | Marlborough, MA 01752, USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please all the division headquarters or refer to our website at
sciex.com/offices